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Differentiation of *Brucella abortus* bv. 1, 2, and 4, *Brucella melitensis*, *Brucella ovis*, and *Brucella suis* bv. 1 by PCR

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Several PCR assays which identify the genus *Brucella* but do not discriminate among species have been reported. We describe a PCR assay that comprises five oligonucleotide primers which can identify selected biovars of four species of *Brucella*. Individual biovars within a species are not differentiated. The assay can identify three biovars (1, 2, and 4) of *B. abortus*, all three biovars of *B. melitensis*, biovar 1 of *B. suis*, and all *B. ovis* biovars. These biovars include all of the *Brucella* species typically isolated from cattle in the United States, a goal of the present research. The assay exploits the polymorphism arising from species-specific localization of the genetic element IS*711* in the *Brucella* chromosome. Identity is determined by the size(s) of the product(s) amplified from primers hybridizing at various distances from the element. The performance of the assay with U.S. field isolates was highly effective. When 107 field isolates were screened by the described method, there was 100% agreement with the identifications made by conventional methods. Six closely related bacteria (*Agrobacterium radiobacter*, *Agrobacterium rhizogenes*, *Ochrobactrum anthropi*, *Rhizobium leguminosarum*, *Rhizobium meliloti*, and *Rhodospirillum rubrum*) and two control bacteria (*Bordetella bronchiseptica* and *Escherichia coli*) tested negative by the assay.

Brucellae are gram-negative bacteria that are pathogenic for humans and a variety of livestock animals and wildlife. The genus *Brucella* has six recognized species on the basis of host specificity. While all six species occur at least sporadically in the United States, the greatest economic impact results from bovine brucellosis caused by *B. abortus*. Infection decreases reproductive efficiency, mainly by abortion. The disease has elicited restrictions in the international an interstate movement of animals. *B. abortus* causes nearly all of the cattle abortions that result from brucellosis. *B. melitensis* (27) and *B. suis* (5, 26) infect cattle and spread within herds but rarely cause abortion (2, 22, 27). Recent evidence indicates that transmission of *B. suis* bv. 1 to cattle by feral swine is on the rise in the United States (26). Thus, while the brucellosis eradication program focuses primarily on *B. abortus*, epidemiologists need to be able to identify both the genus and species of field isolates.

Recent genetic analyses of *Brucella* species (6), including DNA fingerprinting (1, 23), have shown close similarity among the species and have strengthened support for combining *Brucella* strains into a single species (28). This similarity among strains makes identification of *Brucella* species difficult. Because there is little antigenic variation among *Brucella* species, differentiation of species and strains is based on biological and physiological characteristics (phenotype). By analyzing approximately 25 characteristics including susceptibilities to phages, dye sensitivities, and rates of oxidation for a variety of substrates, the genus *Brucella* has been divided into 19 biovars (19). While this technology for specific identification exists, it is time-consuming, expensive, and somewhat hazardous.

PCR is a useful approach for diagnostics (7, 11, 17, 18, 25, 30). It is both quick and inexpensive. Additionally, PCR can use specimens in which the pathogenic organisms have been rendered biologically safe. At least three PCR assays for *Brucella* species have been described (4, 8, 9, 16). These assays are all specific for the genus but not for species. Here we describe a PCR assay that can identify and differentiate most *Brucella* species and biovars found in the United States. It is based on the observations that the repetitive genetic element IS*711* (13) (also previously reported as IS*6501* [24]) is unique to *Brucella* species, and for most species at least one copy of the element occurs at a unique species- or biovar-specific chromosomal locus. The unique locations of these elements are the basis of the diagnostic assay reported here.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in the present study are listed in Table 1. Growth and harvesting of *Brucella* isolates were performed as described previously (14). *Escherichia coli* and *Bordetella bronchiseptica* were grown in Luria broth (20) at 37°C, *Agrobacterium radiobacter* was grown at 30°C, and *Agrobacterium rhizogenes* and *Ochrobactrum anthropi* were grown at 26°C. *Rhizobium meliloti* and *Rhizobium leguminosarum* were grown on Rhizobium X Media (as described in the American Type Culture Collection catalog) at 26°C. All bacteria were killed by the addition of 67% methanol–33% saline (3). *Rhodospirillum rubrum* freeze-dried powder from the American Type Culture Collection was directly suspended and killed in the methanol-saline solution.

Preparation of killed bacteria for PCR. When bacterial cells were used directly for PCR, the killed organisms (see above) were rinsed one time in distilled water to remove the methanol and were then resuspended in distilled water at an optical density of 0.15 to 0.20 at 600 nm (approximately 10^9 cells per ml). The numbers of resuspended bacteria added directly to the PCR mixtures are given in each experiment.

Preparation of genomic DNA. DNA was isolated from *Brucella* species as described previously (12). Briefly, cells were incubated at 50°C for an hour in detergent solution (Zwitter-

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TABLE 1. Bacterial strains used in the study

Organism	Strain designation	Source[a]
Culture collection		
Brucella abortus bv. 1	544	NADC
Brucella abortus bv. 2	2016	NADC
Brucella abortus bv. 3	Tulya	NADC
Brucella abortus bv. 4	292	NADC
Brucella abortus bv. 5	B3196	NADC
Brucella abortus bv. 6	870	NADC
Brucella abortus bv. 7	63/75	NADC
Brucella aboruts bv. 9	C68	NADC
Brucella canis	RM-6/66	NADC
Brucella melitensis bv. 1	16M	NADC
Brucella melitensis bv. 2	63/9	NADC
Brucella melitensis bv. 3	Ether	NADC
Brucella neotomae	SE-1169	NADC
Brucella ovis	ANH3572	NADC
Brucella ovis	ATCC 25840	NADC
Brucella suis bv. 1	1330 (human)	NADC
Brucella suis bv. 2	Danish sow 160	NADC
Brucella suis bv. 3	NADC 710	NADC
Brucella suis bv. 4	IAB 2579	NADC
Brucella suis bv. 5	513	WHO
Escherichia coli	HB101	
Agrobacterium radiobacter	19358	ATCC
Agrobacterium rhizogenes	15834	ATCC
Bordetella bronchiseptica	10580	ATCC
Rhizobium leguminosarum bv. trifolii	14479	ATCC
Rhizobium meliloti	10310	ATCC
Rhodospirillum rubrum	9791	ATCC
Ochrobactrum anthropi	49237	ATCC
Field isolates		
Brucella abortus bv. 1	1001	NADC*
Brucella abortus bv. 1	1003	NADC*
Brucella abortus bv. 1	1005	NADC*
Brucella abortus bv. 1	1006-1009	NADC*
Brucella abortus bv. 1	1011	NADC*
Brucella abortus bv. 1	1019-1036	NADC*
Brucella abortus bv. 1	1059-1061	NADC*
Brucella abortus bv. 1	1038-1057	NADC*
Brucella abortus bv. 1	1064-1070	NADC*
Brucella abortus bv. 1	1073-1087	NADC*
Brucella abortus bv. 1	1089-1090	NADC*
Brucella abortus bv. 1	1104-1106	NADC*
Brucella abortus bv. 1	1092-1095	NADC*
Brucella abortus bv. 1	1098-1102	NADC*
Brucella abortus bv. 1	1146	NADC*
Brucella abortus bv. 1	1148	NADC*
Brucella abortus bv. 1	Strain 19, 1010	NADC*
Brucella abortus bv. 1	Strain 19, 1012	NADC*
Brucella abortus bv. 1	Strain 19, 1091	NADC*
Brucella abortus bv. 2	1062-1063	NADC*
Brucella abortus bv. 2	1088	NADC*
Brucella abortus bv. 2	1096-1097	NADC*
Brucella abortus bv. 4	1013-1014	NADC*
Brucella abortus bv. 4	1037	NADC*
Brucella abortus bv. 4	1058	NADC*
Brucella suis bv. 1	1108	NADC*
Brucella canis	1109	NADC*
Brucella canis	1111-1112	NADC*
Brucella ovis	1113-1114	NADC*
Brucella ovis	1117	NADC*

[a] NADC, National Animal Disease Center; ATCC, American Type Culture Collection; NADC*, field isolates provided to the National Animal Disease Center courtesy of the Diagnostic Bacteriology Laboratory, Animal and Plant Health Inspection Service, U.S. Department of Agriculture. These strains were renumbered when placed in the National Animal Disease Center culture collection.

TABLE 2. Sequences of oligonucleotide primers for PCR

Primer	Sequence (5′–3′)
B. abortus-specific primer	GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC
B. melitensis-specific primer	AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA
B. ovis-specific primer	CGG-GTT-CTG-GCA-CCA-TCG-TCG
B. suis-specific primer	GCG-CGG-TTT-TCT-GAA-GGT-TCA-GG
IS*711*-specific primer	TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT
IR-1	GGC-GTG-TCT-GCA-TTC-AAC-G
IR-2	GGC-TTG-TCT-GCA-TTC-AAG-G
rRNA-1	AGA-GTT-TGA-TCC-TGG-CTC-AG
rRNA-2	ACG-GCT-ACC-TTG-TTA-CGA-CTT

gent 3-14, 1%) containing 0.1 M citric acid. The cells were washed in TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) and were then lysed by the sequential addition of lysozyme, proteinase K, EDTA, and sarcosine. After treatment with RNase A, the DNA was extracted with phenol and precipitated with ethanol. The DNA, dissolved in TE, was purified by gradient centrifugation in cesium chloride.

Southern analysis and probe preparation. Genomic DNAs (350 ng each) were digested with *Eco*RI. The resulting fragments were separated by electrophoresis on a 0.8% agarose gel in 1× TBE (100 mM Tris, 90 mM boric acid, 1 mM EDTA [pH 8.4]), denatured (0.5 M NaOH, 1.5 M NaCl), neutralized (0.5 M Tris, 3 M NaCl), and blotted onto a nylon membrane (Nytran; Schleicher & Schuell, Keene, N.H.) in 20× SSC (3 M NaCl, 0.3 M sodium citrate [pH 7.0]) overnight. The blot was dried and baked at 80°C for 2 h before prehybridization in aqueous hybridization buffer (0.1 M Tris [pH 8.0], 0.5 M NaCl, 2 mM EDTA, 1% sodium dodecyl sulfate [SDS]) containing 0.5 mg of denatured, sheared, calf thymus DNA per ml for 3 h. The probe was prepared by 35 cycles of PCR amplification (see below) of cloned IS*711* (pBO31-I1 [14]) by using IR-1 and IR-2 as primers (Table 2). Approximately 100 ng of amplified product was radiolabeled by random hexamer priming in the presence of [α-^{32}P]dCTP according to the manufacturer's instructions (catalog no. 1004 760; Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The labeled product was heat denatured in a boiling water bath for 5 min and was then added to 40 ml of aqueous hybridization buffer containing 0.2 mg of calf thymus DNA per ml. Hybridization was performed overnight at 42°C, after which the blot was washed repeatedly in decreasing-ionic-strength buffers, ending in a 30-min wash in 0.16× SSC–0.1% SDS at 65°C. Autoradiography onto X-AR film was performed at −70°C for 24 h in the presence of an intensifying screen.

Cloning of IS*711* elements and the selection of PCR primers. Southern blot analysis to determine the distribution of IS*711* in the six *Brucella* species showed the presence of at least one potentially unique copy of the element in every species except *B. canis*. Biovars of interest to the project were analyzed with several restriction enzymes to identify which enzyme resulted in the best separation of one unique copy from each species (data not shown). The selected enzyme was then used for cloning the uniquely sized fragment from a given species. A *Hin*dIII genomic library of *B. abortus* 544, a *Cla*I genomic library of *B. melitensis* 16M, and an *Eco*RI library of *B. suis* 1330 were constructed by digesting genomic DNAs with the appropriate restriction enzyme and ligating the fragments into pUC12 (gift from J. Messing) linearized with the same restriction enzyme (or *Acc*I for the *Cla*I library). Calcium chloride-treated *E. coli* (XL1-Blue; Stratagene, La Jolla, Calif.) was

transformed with the respective ligation mixtures. Transformants were selected by plating onto medium (LB agar [20]) containing ampicillin and were blotted onto nylon membranes (Nytran). Bacteria were lysed by wetting the membrane with 2× SSC containing 5% SDS and then heating the wet membrane for 3 min in a microwave oven (10). The colonies were screened by hybridization of radiolabeled IS*711* DNA (see above) to the membrane-bound DNAs as described above for the Southern hybridizations.

Colonies which hybridized with the IS*711* probe were selected, their DNAs were isolated and digested with the appropriate restriction enzyme, and the fragments were separated by electrophoresis. The sizes of the inserts were compared with those on Southern blots, and the fragments which appeared to contain the unique copy were analyzed. The resulting plasmids and their respective insert sizes are given in Table 3.

The DNA flanking the 3′ end (relative to the published sequence [13]) of each IS*711* copy selected was sequenced with [α-^{35}S-thio]dATP by the dideoxy method of Sanger according to the manufacturer's instructions (Sequenase Version 2.0; U.S. Biochemicals, Cleveland, Ohio). For PCR, short sequences (21 to 24 bp in size) at selected distances from the insertion sequence element were chosen by using the Oligo version 3.4 computer program (National Biosciences, Hamel, Minn.). The Oligo program also selected a short DNA sequence from inside the BCSP31K gene (21) upstream of IS*711* of *B. ovis* (14) and a sequence from within IS*711* (13) on the basis of published sequence data.

TABLE 3. Clones containing IS*711* copies at unique loci

Species	Restriction library	Plasmid designation[a]	Insert size (kb)
B. abortus	*Hin*dIII	pBA-PCR-1L	4.3
B. melitensis	*Cla*I	pBM-PCR-M3	7
B. ovis	*Eco*RI	pBO-31-I1[b]	5.1
B. suis	*Eco*RI	pBS-PCR-S12	4.4

[a] Plasmid constructions are described in Materials and Methods.
[b] Construction of this plasmid has been described previously (14).

Primers. Oligonucleotide primers were synthesized at the National Animal Disease Center or at the Nucleic Acid Facility at Iowa State University, Ames. The *Brucella* PCR diagnostic assay primer cocktail is composed of the five primers described in Table 2. The other PCR primers used in the study are also listed in Table 2.

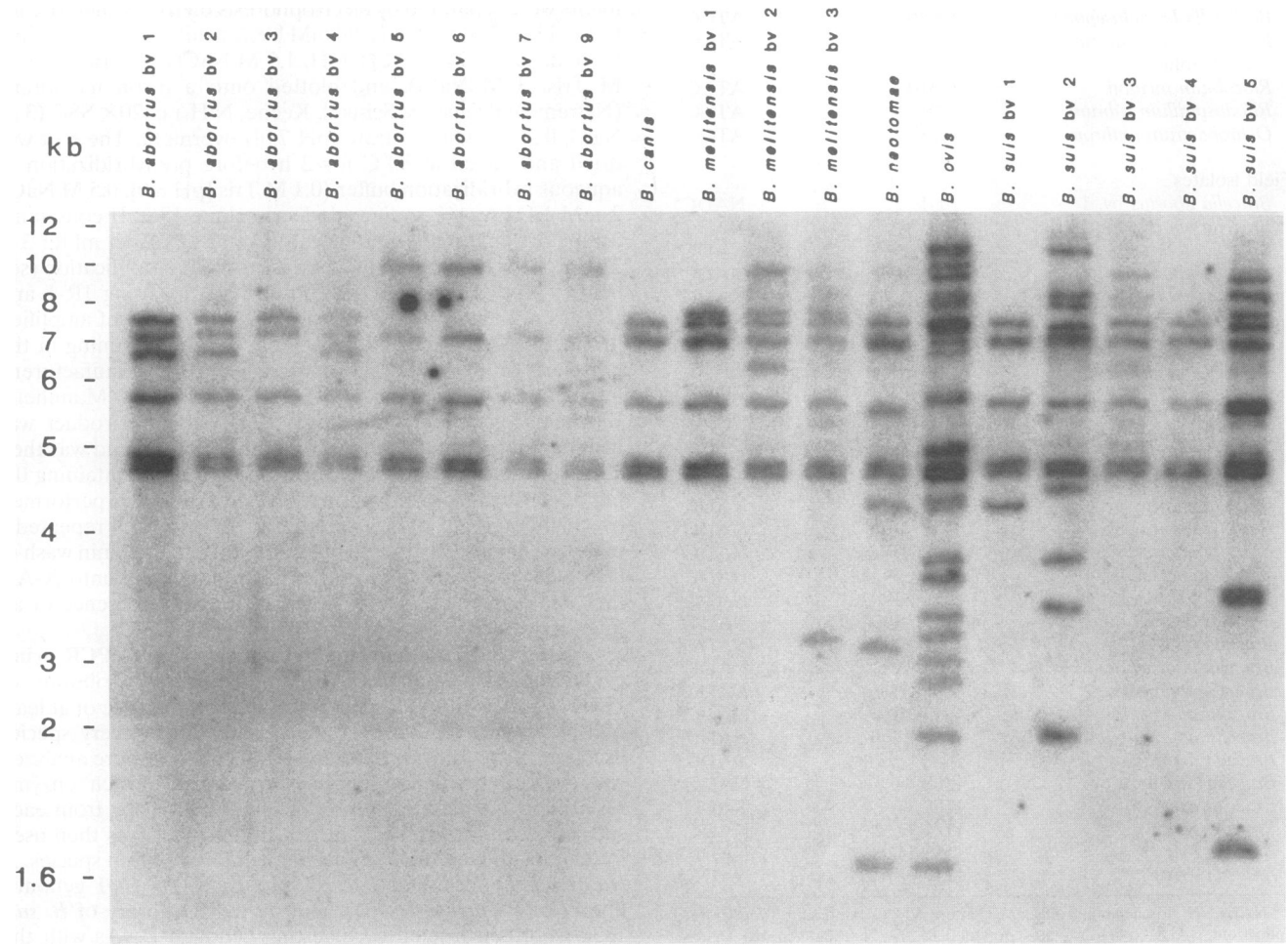

FIG. 1. Southern blot analyses of genomic DNAs probed with IS*711*. Genomic DNAs (350 ng each) of six species encompassing the 19 biovars of *Brucella* were digested with *Eco*RI and were resolved by agarose gel electrophoresis. After transfer to a nylon membrane, the DNAs were probed with ^{32}P-labeled IS*711* and autoradiographed. The species and biovar designations are listed above the lanes. The mobilities of the size standards are listed along the left side of the blot.

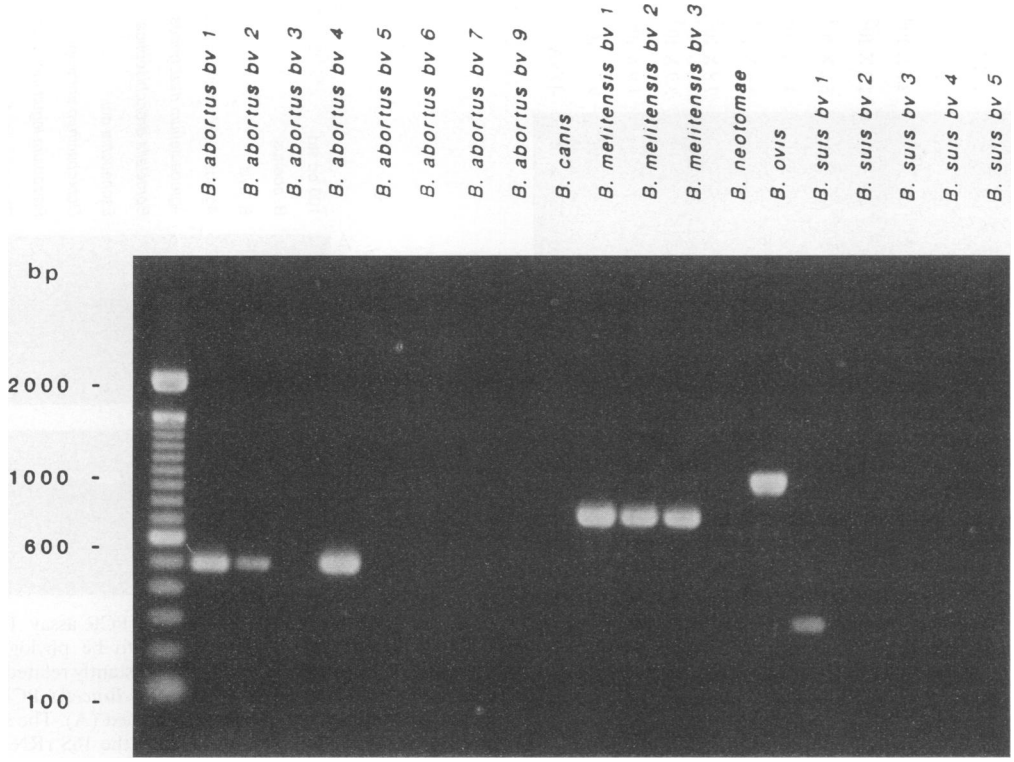

FIG. 2. PCR products amplified from 19 *Brucella* biovars. Genomic DNAs from 19 *Brucella* biovars were tested in the PCR assay as described in the text. A negative control of water containing no target DNA was also included in the assay. Eight microliters of each reaction mixture was resolved by agarose gel electrophoresis, and the amplified products were stained and photographed. The species and biovar designations are listed above the lanes. The first lane contains a 100-bp ladder as a size standard.

PCR assay. PCR assay conditions were determined by using the PCR optimization kit (according to the manufacturer's instructions [In Vitrogen, San Diego, Calif.]). The reaction mixture consisted of 60 mM Tris-HCl (pH 9.0), 15 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 250 μM (each) the four deoxynucleoside triphosphates (dNTPs), and the five-primer cocktail (0.2 μM [each] *B. abortus*-, *B. melitensis*-, *B. ovis*-, and *B. suis*-specific primers and 1 μM IS*711*-specific primer [Table 2]). One unit of *Taq* polymerase per 45-μl reaction mixture was added before the reaction mixture was dispensed into MicroAmp vials (22.5 or 45 μl per tube; GeneAmp; Perkin-Elmer Cetus, Norwalk, Conn.). For assays involving purified DNAs, 5 μl per 50-μl reaction mixture of target DNA ($\sim 5 \times 10^5$ copies of genomic DNA or $\sim 2 \times 10^7$ copies of plasmid DNA) was added. When killed bacterial cells were used, approximately 2×10^6 organisms (in 2.5 μl of water) were added per 25-μl reaction mixture unless otherwise stated. The samples were cycled (1.15 min at 95°C, 2.0 min at 55.5°C, 2.0 min at 72°C) 35 times in a thermocycler (Perkin-Elmer GeneAmp PCR System 9600). After the last cycle, the reaction mixtures were incubated for an additional 5 min at 72°C before they were stored at 4°C. The products (5 to 8 μl from each reaction mixture) were analyzed by electrophoresis through a 1.5% agarose gel, after which the gel was stained with ethidium bromide and photographed.

Nucleic acid amplification of DNA from killed bacterial cells other than *Brucella* species was performed as described above. PCR of the 16S rRNA gene (29) was performed in a reaction mixture consisting of 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, the four dNTPs (200 μM each), and 1.25 U of *Taq* polymerase per 50-μl assay mixture according to the manufacturer's instructions (catalog no. N801-0043; GeneAmp DNA Amplification Reagent Kit; Perkin-Elmer Cetus).

RESULTS

Distribution of IS*711* in *Brucella* species. The copy number and distribution of IS*711* in *Brucella* species were determined by Southern blot analysis of *Eco*RI-digested genomic DNA probed with radiolabeled IS*711* (Fig. 1). Each species has at least five copies of IS*711*. Several copies appear to be at common loci in all six species. With the exceptions of *B. abortus* bv. 3, *B. canis*, and *B. suis* bv. 4, which appear to have identical hybridization patterns (Fig. 1), all biovars could be identified as to species by the hybridization patterns. However, biovars within a species cannot always be distinguished by the IS*711* restriction fragment length patterns generated by Southern blot analysis (e.g., *B. abortus* bv. 1, 2, and 4 have identical hybridization patterns.)

Amplification of DNA fragments from the 19 *Brucella* biovars by PCR with a primer cocktail. A five-primer cocktail was used to amplify DNA fragments. Purified genomic DNAs (5 ng/50-μl assay mixture) from each of the 19 *Brucella* biovars (Table 1) were evaluated as targets. DNA was amplified from four *Brucella* species (Fig. 2), including *B. abortus* bv. 1, 2, and 4 (498 bp); *B. melitensis* bv. 1, 2, and 3 (731 bp); *B. ovis* (976 bp); and *B. suis* bv. 1 (285 bp). The other species were not expected to produce products. Some primer-dimer complexes of less than 100 bp in size were also observed.

Sensitivity of the assay. Decreasing numbers of bacteria (methanol killed and washed but not lysed) were added to the reaction mixture to determine the sensitivity of the assay. The

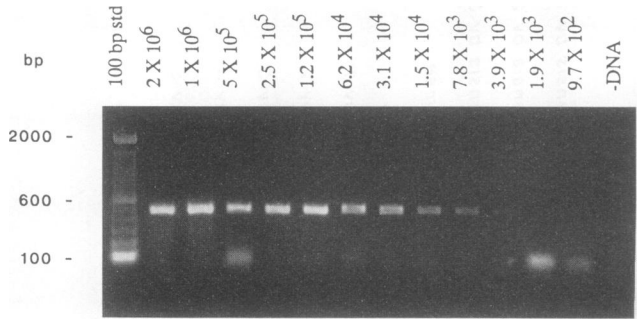

FIG. 3. Sensitivity of the PCR assay with *B. abortus* cells. Killed *B. abortus* field strain 1031 (~1.0×10^9 bacteria per ml) was serially diluted and assayed under standard conditions as described in the text. A negative control of water containing no bacteria was also included in the assay. Eight microliters of each product was separated by gel electrophoresis, stained in the presence of ethidium bromide, and photographed. The numbers of bacteria per 25 μl assay mixture are listed above the lanes. The first lane contains a 100-bp ladder as a size standard.

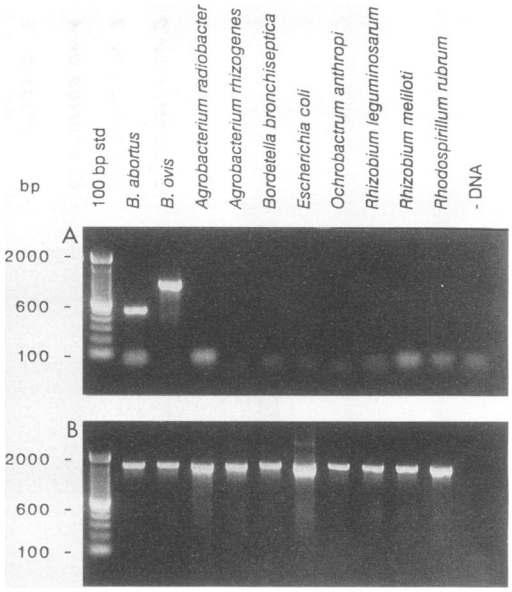

FIG. 4. Specificity of the *Brucella* PCR assay. Bacterial cells from four genera (six species) reported to be phylogenetic relatives of *Brucella* species and from two more distantly related genera (*Bordetella* and *Escherichia*) were tested by the *Brucella* PCR assay under the standard conditions described in the text (A). The same bacteria were amplified with conserved primers for the 16S rRNA genes to demonstrate that the quantity and quality of the DNAs were suitable for PCR amplification (B). In both assays the field strains (*B. abortus*) 1031 and (*B. ovis*) 1-507 were included as positive controls. A negative control of water containing no bacteria was also included in each assay. The genus and species identifications are listed above the lanes. The first lane in each panel contains a 100-bp ladder as a size standard. Products of less than 100 bp were presumed to be primer-dimer complexes.

results (Fig. 3) indicate that the assay can be used with a wide range of target concentrations. Under the conditions established for the assay, 7,000 bacteria were consistently detected in a 25-μl reaction mixture even when the bacteria used were stored in methanol-saline for more than a year. Colleagues from the Veterinary Sero-Epidemiology Laboratory at Colorado State University have detected 10^2 *B. melitensis* by the standard assay (25a). The use of fresher specimens and minor adjustments in assay conditions should increase the assay's sensitivity.

The conditions under which the assay is performed are critical with the five-primer cocktail. The results obtained from using the PCR Optimization Kit (InVitrogen) indicated that the assay is highly sensitive to the $MgCl_2$ concentration (optimum, 1.5 mM) and the pH (optimum, 9.0) of the reaction buffer (data not shown). The assay was not affected when the primers or target DNA contained TE, as long as the total volume of TE did not exceed 10% of the final sample volume. The effects of using alternative reaction conditions were either the appearance of false products of less than 300 bp or the lack of DNA amplification in the presence of the target. Primer-dimer complexes of less than 100 bp were occasionally observed in the reaction mixtures, especially when little or no target DNA was present.

Effect of foreign DNA on the assay. The identification of *Brucella* phenotypes by conventional methods is dependent on the isolation of pure strains of *Brucella*. Contamination by other bacteria can occur in the tissues used to isolate *Brucella*. This necessitates an extra step to remove these other bacteria. The effects on the PCR diagnostic assay of *E. coli* genomic DNA in the target sample were investigated. When various amounts of *E. coli* DNA (including an amount equivalent to the target DNA) were added to the target DNA sample, the amplification reaction was unaffected (data not shown). The specificity of the assay was examined by testing *A. radiobacter*, *A. rhizogenes*, *B. bronchiseptica*, *E. coli*, *O. anthropi*, *R. leguminosarum*, *R. meliloti*, and *R. rubrum* DNAs as targets. All of the bacteria tested were negative under the assay conditions described above (Fig. 4). The same DNAs were effectively amplified by using common 16S rRNA primers to confirm that the reactions were devoid of PCR inhibitors (Fig. 4).

Survey of *Brucella* field isolates. Isolates from throughout the United States are routinely sent to the Diagnostic Bacteriology Laboratory (Animal and Plant Health Inspection Service, U.S. Department of Agriculture) in Ames, Iowa, for identification or verification. After identification of the genus, species, and biovar by conventional methodology, 107 randomly selected isolates of *Brucella* species were given to our laboratory for identification by PCR. The isolates were grown and then killed in methanol-saline as described above. Approximately 2×10^6 washed bacteria were added directly to 22.5 μl of the PCR mixture. For each group of isolates tested, a DNA-free control was included as a control for contamination with target or product. No contamination by target DNA or amplified product was ever observed. The resulting products are summarized in Table 4. Figure 5 shows the results of PCR amplification of DNAs from 18 of these isolates. In all cases

TABLE 4. Results for field strains tested by PCR[a]

Species and biovar	No. of strains assayed	Fragment size observed (bp)
B. abortus bv. 1	88	500
B. abortus bv. 1, strain 19	3	500
B. abortus bv. 2	5	500
B. abortus bv. 4	4	500
B. canis	3	None
B. ovis	3	1,000
B. suis bv. 1	1	285

[a] Results for all strains correlated 100% with the results obtained by the Diagnostic Bacteriology Laboratory.

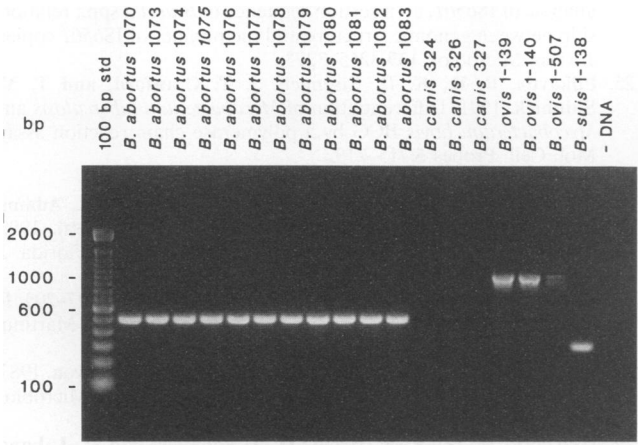

FIG. 5. PCR products amplified from 18 *Brucella* field isolates. Shown are the products amplified from 18 of the field isolates (~2 × 10^6 bacteria) tested by the PCR assay described in the text. The strains shown are from samples from within the United States sent to the Diagnostic Bacteriology Laboratory for testing and encompass the species and biovars found in the United States. A negative control of water containing no bacteria was also included in the assay. Reaction mixtures were resolved by agarose gel electrophoresis, and the amplified products were stained and photographed. The strain designations are listed above the lanes. The first lane contains a 100-bp ladder as a size standard.

the PCR assay agreed with the identification determined by the Diagnostic Bacteriology Laboratory.

DISCUSSION

We developed a PCR assay that can identify and differentiate most *Brucella* species and biovars found in the United States, including all types usually found in cattle (15). It is based on the observation that the genetic element IS*711* occurs at several species-specific or biovar-specific chromosomal loci. The assay was designed to amplify species-specific-sized products by using five primers, one of which hybridizes to the IS*711* element and the others of which hybridize to one of four species-specific regions adjacent to the element. Thus, the products are composed of a portion (281 bp) of the IS*711* element and a predetermined number of nucleotides flanking the 3′ end of the element at a species-specific (or biovar-specific) locus.

Field strains of *B. abortus* amplified a 498-bp DNA fragment. While only three of the eight biovars of *B. abortus* are identified by the assay, these three are the only biovars found in the United States (7a, 15). All three biovars of *B. melitensis* were identified by amplification of a 731-bp fragment. Only one biovar of *B. suis*, bv. 1, was identified by amplification of a 285-bp fragment; however, this is the most prevalent biovar found in the United States and is the only biovar of *B. suis* reported in cattle from the United States (7a, 15). *B. ovis* has a single biovar, and the three isolates tested were identified correctly on the basis of amplification of a 976-bp fragment. Thus, when 107 randomly selected isolates of *Brucella* from the United States were tested, all 107 gave the predicated results.

The assay described in this report has several advantages over the current microbiological methods used to identify *Brucella* species. A major advantage is the speed with which the assay can be performed, i.e., less than a single working day. Conventional methods require at least several days. Because isolation of the DNA is not necessary, another major advantage is the minimal sample preparation. As few as 10^4 bacteria can be added directly to the reaction mixture. Live *Brucella* organisms are not necessary for the assay. This is significant because *B. abortus* is a human pathogen. It is slightly less expensive to perform the PCR assay than the conventional methods in use. Because the assay is very amenable to automation and robotics, the costs could be reduced even further. Finally, the assay is unaffected by contamination by other microbes that might be present in the tissue samples used for isolation. After collection, the bacteria can be killed and sent for identification. The main disadvantage of the assay lies in our present inability to differentiate the vaccine strain from field isolates. Once this obstacle is overcome, this assay will sufficiently identify the agents of bovine brucellosis in the United States.

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